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W.W. Wagoire · R. Ortiz · J. Hill · O. Stølen

Comparison of methods for calculating the heritability of adult field resistance to yellow rust and grain yield in spring wheat

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Abstract Several methods are available for estimating heritability in disomic species, including parent-offspring regression, realized heritability, intraclass correlations of recombinant inbred lines, and diallel-cross analysis. Estimates were obtained by these various methods for a set of eight bread wheat (*Triticum aestivum*) lines adapted to the East African highlands, which had been intercrossed and selfed in a half-diallel arrangement to give F_1, F_2 and F_3 generations, and F_6 recombinant inbred lines. Significant genetic variation existed among parents and crosses for both grain yield and yellow rust resistance in all generations. Based on the heritability calculated from the analysis of F_6 recombinant inbred lines, analysis of the F_2 diallel crosses was recommended for determining the heritability of both characters in early segregating generations. The results also suggest that a form of tandem selection may be effective in developing locally adapted germplasm which combines high grain yield with yellow rust resistance.

Key words *Triticum aestivum* · Diallel cross · Parent-offspring regression · Realized heritability · Yellow rust · Yield

Introduction

Plant breeders work with continuously varying or quantitative characters, which are controlled by genes whose individual effects are too small to be detected by conventional Mendelian analysis. Hence, the properties and actions of these genes have to be inferred from analyses of the means and variances of appropriate generations or populations (Mather and Jinks 1982; Falconer and Mackay 1996; Kearsey and Pooni 1996; Hill et al. 1998). Information obtained from these analyses can be used to determine in which generation selection should be practised; what the consequences of inbreeding are; what the genetic basis of heterosis is, and whether inbred or hybrid cultivars should be developed? Thus, if a particular character is controlled by recessive genes, selection should be delayed until the generations are more homozygous. If heritability is low then either increased replication is required, or else selection for a correlated character may be practised to increase the chances of identifying superior genotypes (Hill et al. 1999). Dispersion of unidirectionally dominant genes among parental lines, leading to better parent heterosis for the character in the F_1, could be exploited by developing hybrid cultivars. This strategy could also be used during the initial phase of a breeding programme designed to identify those crosses from which a high proportion of superior recombinant inbred lines are expected (Lawrence and Senadhira 1998).

Different mating designs have been suggested for calculating the heritability of populations derived from inbred lines in disomic hexaploid wheat (*Triticum aestivum*). Parent-offspring regression and diallel crosses are among the most popular. Alternatively, realized heritability and intraclass correlations, calculated from recombinant inbred lines derived by single-seed descent, may be used. This paper compares these methods of calculating the heritability of grain yield and resistance to yellow rust, caused by *Puccinia striiformis* Westend., in bread wheat germplasm adapted to the East African highlands.

Materials and methods

Eight wheat lines were selected as parents for this experiment on the basis of their grain yield and resistance to yellow rust at Kalengyere, a high-rust site in the south western highlands of Uganda (Table 1). These eight lines were crossed in a half-diallel

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W.W. Wagoire · R. Ortiz (✉) · J. Hill · O. Stølen
Department of Agricultural Sciences,
The Royal Veterinary and Agricultural University,
40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen,
Denmark

Present address:
R. Ortiz, ICRISAT, Patancheru 502 324, Andhra Pradesh, India
e-mail: rortiz@cgiar.org
Fax: +91 40 241239

Table 1 Mean yellow rust cofficient of infection (CI) and grain yield (g m^{-2}) of the eight parental lines

Code	Parent name/pedigree	CI	Grain yield
1	BURI CM58340-A-1Y-2M-2Y-0M	0.25	130.00
2	K. CHIRIKU K. TEMBO/CARPINTERO″S″	0.20	103.67
3	ESDA/LIRA CM78428-017M-013M-013Y-03AL-3Y-3AL-0Y	0.00	150.87
4	VEE″S″/JUP73/EMU″S″//GJO″S″ CM74465-05AP-300AP-4AP-300AL-0AP	5.00	131.90
5	ATTILA CM85836-4Y-0M-0Y-OPZ	19.50	91.77
6	CY8801	45.00	53.00
7	F60314.76/4CNO76/7C//KAL/BB/3/PC1″S″/5/CNO79	35.00	16.63
8	CAR853/COC//VEE″S″/3/E7408/PAM″S″/HORK″S″/PF73226	70.00	4.83
Mean square error		143.32	861.25

Table 2 Means for yellow rust coefficient of infection (CI) and grain yield (g m^{-2}) for F_1, F_2, F_3 and F_6 single-seed descent lines (SSD) of an 8×8 half diallel among bread wheat lines

Cross	Yellow rust (CI)				Grain yield (g m^{-2})			
	F_1	F_2	F_3	F_6SSD	F_1	F_2	F_3	F_6SSD
1×2	0.50	0.20	0.20		178.00	164.7	131.7	
1×3 (21)[a]	2.15	2.38	1.14	24.44	163.27	180.4	164.0	55.90
1×4	1.10	2.77	1.33		97.15	125.8	153.5	
1×5 (11)	2.10	3.47	14.96	36.86	95.53	79.0	106.2	33.65
1×6 (14)	3.00	4.91	3.21	9.98	94.60	123.7	111.2	75.21
1×7 (12)	4.00	6.36	9.47	6.80	66.30	57.2	62.4	119.57
1×8	12.00	17.44	8.93		54.37	57.3	42.5	
2×3 (13)	0.10	0.05	3.23	14.59	112.07	132.5	155.2	83.09
2×4 (19)	1.90	2.07	0.96	0.88	74.18	82.3	97.0	101.92
2×5 (16)	1.40	4.40	12.30	18.00	97.67	110.3	102.4	69.65
2×6 (12)	1.40	8.12	6.78	15.56	162.03	127.2	128.8	74.20
2×7 (11)	2.00	10.27	17.30	19.65	118.70	80.4	40.1	96.40
2×8 (10)	3.00	10.20	0.60	22.59	115.27	109.4	98.3	62.41
3×4 (11)	0.20	2.87	2.09	0.62	334.00	84.8	176.2	131.87
3×5 (31)	0.60	1.97	7.03	17.24	183.47	89.4	82.8	95.17
3×6 (12)	1.20	8.31	5.95	5.17	143.53	175.6	124.4	108.14
3×7 (10)	0.50	6.13	7.06	50.24	129.70	129.8	111.4	21.91
3×8 (11)	0.50	6.65	0.20	1.42	190.80	75.9	160.6	93.40
4×5 (12)	38.00	17.20	9.77	16.86	108.33	95.0	122.0	81.29
4×6 (15)	9.41	7.01	7.40	9.67	151.10	69.0	90.6	92.34
4×7 (17)	45.04	13.52	10.77	9.74	63.60	47.3	94.1	94.43
4×8 (10)	23.00	37.39	43.57	2.39	57.27	43.3	53.3	142.42
5×6 (11)	30.00	12.54	10.00	29.01	58.23	66.4	55.5	40.34
5×7	23.00	28.64	9.10		72.77	38.5	29.7	
5×8	70.00	23.92	22.70		25.70	52.9	66.4	
6×7	70.00	49.13	52.07		27.73	38.6	10.7	
6×8	50.00	32.42	15.86		54.50	62.8	65.5	
7×8	70.00	46.81	9.00		10.53	65.0	36.4	
Mean square error	98.65	71.60	31.92		1955.11	795.70	1020.80	

[a] Number of F_6SSD lines per cross combination indicated in brackets

mating design to produce 28 F_1 hybrids. Reciprocals were not included because neither maternal effects nor reciprocal differences affected either character significantly at the test site (Wagoire et al. 1998a, b). These 28 F_1s were selfed to obtain the corresponding F_2 populations. Similarly, random plants from each F_2 population were selfed and the seed bulked to produce the F_3 generation for each of the 28 cross combinations. A total of 279 F_6 recombinant inbred lines (RILs) were derived from 20 of the 28 possible cross combinations after selfing individual plants for five generations using the single-seed descent (SSD) method (Brim 1982). The number of RILs per cross combination ranged from 10 to 31. Although the RILs were developed in a rust-free environment, most of the missing lines were from crosses in which at least one parent was susceptible to yellow rust (Table 2).

The experiment was conducted at Kalengyere, located 2460 m above sea level, which has an annual temperature of 16°C, and an average rainfall of 750 mm during the trial period. The soil at the experimental site was an andosol with a pH of 5.7. A randomized complete block design with two replications was used for this trial. Spacing between plants within plots was 0.15 m, while nitrogen was applied at planting at a rate of 50 kg ha^{-1}.

Yellow rust was scored on the flag leaf of individual plants when the severity on the most susceptible parent was about 100%, i.e. most of the leaf surface was covered with uredinia. The modified Cobb scale (Peterson et al. 1948) was used for scoring the percentage of possible tissue rusted (disease severity). Host-response to infection was scored using T (=0.1) for immune plants; R (=0.2) for resistant plants showing miniature uredinia; MR

Table 3 Mean squares for F_1, F_2, and F_3 generations using Griffing's diallel-analysis method

Source of variation	*df*	Yellow rust			Grain yield (gm^{-1})		
		F_1	F_2	F_3	F_1	F_2	F_3
Replications	1	391.91	173.76	194.16	16,167.01**	3,240.13	1,641.65
Genotypes	35	713.81***	593.02***	538.82***	8,162.78***	4,105.32***	4,359.39***
GCA	7	2,741.98***	2,282.60***	1,324.80***	23,993.48***	15,202.26***	15,490.72***
SCA	28	206.78	170.62	342.32**	4,205.10***	1,331.10	1,576.56
Error	35	69.52	88.54	57.47	1,671.67	843.95	1,054.28
Narrow-sense heritability		0.60	0.63	0.30	0.37	0.60	0.57

Table 4 Narrow-sense heritability calculated by parent-offspring regression; b=regression slope, r=coefficient correlation

Parent-offspring	Yellow rust (CI)		Grain yield (gm^{-2})	
	b	r	b	r
Pi-F_1	1.013±0.207***	0.693	1.283±0.284***	0.664
F_1–F_2	0.493±0.061***	0.844	0.290±0.106***	0.471
F_2–F_3	0.633±0.110***	0.730	0.779±0.153***	0.706

Table 5 Narrow-sense heritability based on response to a hypothetical selection intensity of 20%

Generation	Yellow rust (CI)	Grain yield (gm^{-2})
F_2	0.484	0.403
F_3	0.664	0.861

(=0.4) for moderately resistant plants exhibiting small uredinia; MS (=0.8) for moderately susceptible plants with moderate sized uredinia (smaller than the fully susceptible type); and S (=1) for fully susceptible plants. Disease severity and host response scores were multiplied together to give the coefficient of infection (C.I.) for data analysis. Whole plots were hand-harvested, threshed, cleaned, sun-dried and the grain weighed at approximately 12% moisture content. Plot grain yields were expressed as g m^{-2}.

Analyses of variance were carried out on plot means for both characters. Parent-offspring regressions and correlations were calculated as indicated by Nyquist (1991) or Falconer and Mackay (1996). Heritability was also estimated on an entry mean basis, using the diallel analysis of Griffing (experimental method 2, 1956), and from the intraclass correlation after a nested analysis of variance of the F_6 RIL (Kearsey and Pooni 1996). Realized heritability (RH) was calculated as the mean difference between the highest and lowest crosses in each generation, divided by the corresponding mean difference in the previous generation (Fehr 1987). Thus, for F_2-F_1, the difference between these crosses in the F_1 would be equivalent to the selection differential (S), the same difference in the F_2 being equivalent to the response to selection (R). The hypothetical intensity of selection used to calculate RH in these simulations was 20%.

Results

Significant differences existed among genotypes within all generations for both host response to yellow rust and grain yield (Tables 2 and 3). Diallel analysis confirmed the significance of general combining ability (GCA) for both characters in this germplasm. Specific combining ability (SCA) was only significant for yellow rust resistance in the F_3 generation and grain yield in the F_1, which would explain the low narrow-sense heritabilities recorded in these generations (Table 3).

Parent-offspring regressions were highly significant for both yellow rust resistance and grain yield in the three consecutive generations (Table 4). They were not significant for either character between the F_3 generation means and the mean of the RILs derived from the same cross (data not shown). Realized heritability was higher for yellow rust than for grain yield for the F_2-F_1 simulated selection, while the reverse was true for the F_3-F_2 simulated selection (Table 5).

Significant differences existed among and within those 20 crosses with RILs for both yellow rust and grain yield (Table 6). The most-resistant RIL population was derived from a cross between the most-resistant line (Esda/Lira, 0% yellow rust) and the partially resistant line 4 (5% yellow rust). Similarly, the RIL with the highest mean grain yield arose from a cross between the partially resistant line 4 and the most-susceptible line 8 (70% yellow rust), which demonstrates the feasibility of transferring an adequate level of yellow rust resistance (2.4%) by conventional cross breeding. The highest-yielding individual RIL (283 g m^{-2}) was derived from the cross between Esda/Lira and line 4.

Discussion

The heritability of a given trait depends on the material under test, the environment in which the material is being grown, and the experimental unit – plant, plot or replicated trial – used to measure the phenotypic expression of the trait (Hill et al. 1998). As Hanson (1963) points out, heritability is that 'fraction of the selection differential expected to be gained when selection is practised on a defined reference unit'. The resultant estimates may have large errors associated with them. Consequently, published estimates of heritability for the same trait in the same crop may differ widely. Nevertheless, as a concept heritability can supply plant breeders with useful in-

Table 6 Hierarchial analysis of variance of F_6 single-seed descent lines

Source of variation	Yellow rust (CI)		Grain yield (gm^{-2})	
	df	MS	*df*	MS
Replications	1	2,293.725***	1	81,846.190***
Genotypes	278	801.115***	278	6,877.580***
Crosses	19	3,763.270***	19	21,953.834**
F_6SSD/crosses	259	583.813***	259	5,771.649**
Error	277	108.996	272	2,181.120
Variance between crosses		114.740		583.984
Variance among SSD within crosses		237.408		1,695.224
Intraclass correlation		0.326		0.257
Narrow-sense heritability		0.652		0.515

formation about the selection potential of their material, but it can be both misunderstood and misused.

Estimates of heritability calculated from midparent-F_1 offspring regression were not significantly different from 1 in this experiment for either yellow rust or grain yield (Table 4). This method of determining heritability may be biased, however, because of environmental covariances (Falconer and Mackay 1996). When parents and offspring are tested together in the same experiment, their covariance depends not only on the covariance between genotype and environment, but also on the environmental covariance between parent and offspring (Vogel et al. 1980). The covariance between genotype and environment may be zero if the parents and offspring are randomized independently, but the environmental covariance remains as a source for inflating these estimates of heritability. Furthermore, non-zero genotype-environment interactions also affect heritability estimates when parents and offspring are assessed in the same environment (Casler 1982). Estimates of heritability calculated from parent-offspring regression may therefore be inflated, and this will give rise to misguided optimism when calculating the genetic advance expected through selection. Frey and Horner (1957) suggest parent-offspring correlation as an alternative for computing heritability. But as Falconer and Mackay (1996) point out this correlation may be affected by the number of parents and offspring, and, unlike the regression slope, the correlation bears no simple relationship to the causal components of variance.

It is of interest to compare heritability estimated from F_6 RIL, when genotypes are almost homozygous, with estimates from the early, heterozygous generations in the selfing series. Thus, an estimate obtained from the intraclass correlation of the F_6 SSD RIL for yellow rust (Table 6) is very similar to that calculated from the analysis of F_1 and F_2 diallel crosses (Table 3), and F_2-F_3 realized heritability (Table 5). Furthermore, heritability for grain yield in the F_6 SSD RIL is not significantly different from estimates calculated from the analysis of F_2 and F_3 diallel crosses. These results suggest that heritability estimated from an F_2 diallel is as reliable as those from other generations, especially those affected by significant specific combining-ability effects; for example, grain yield in the F_1, or the shift towards yellow rust resistance in the F_3 generation derived from some crosses between susceptible parents (Table 2). Use of the F_2 generation may also alleviate those problems arising from insufficient F_1 seed and enable replicated trials across locations to be conducted (Christie and Shattuk 1992). Jinks (1956) provides an in-depth analysis of an F_2 diallel cross.

Estimates of heritability calculated from selection experiments are referred to as realized heritability (RH), because they measure directly the expected response to a given selection intensity. Furthermore, a comparison of RH with other methods of calculating heritability allows potential bias in this calculation to be detected. As indicated by Falconer and Mackay (1996), RH may provide a biased heritability estimate for a population because of changes unrelated to selection in the reference population, such as systematic shifts due to environmental effects, inbreeding depression, or random drift. The values shown in Table 5, suggest that this may have happened for the F_2-F_1 RH, and for grain yield in the F_3-F_2 RH. Overall, however, the various estimates of heritability obtained here might be considered rather high, and are perhaps not typical of elite plant-breeding material. This result is due to the large variation exhibited by the parental lines for both characters (Table 1), which stems from the need to use a broad-based population as the source material, while conducting the trial at a high rust site merely accentuates the parental differences (Wagoire et al. 1999).

In the highlands of Uganda, yield loss resulting from yellow rust alone has been calculated to be 25% (Wagoire et al. 1998a). Yellow rust may be easily scored on individual plants, while the assessment of grain yield requires larger plots and is more time-consuming. Moreover, earlier work has already established that at Kalengyere indirect selection for yield via improved yellow rust resistance may be more effective than direct selection for yield itself (Hill et al. 1999). Thus a tandem selection scheme could be practised, whereby individual F_2 plants resistant to yellow rust are selected as parents for the development of an F_6 SSD RIL, particularly when such individuals are islands of resistance in a sea of susceptibility. These RILs may then be evaluated for grain yield in replicated trials. Genotypes combining high yield with yellow rust resistance could be tested further in multilocational trials, with a view to developing bread wheat cultivars adapted to the East African highlands.

An off-season nursery could be used to produce these RILs, thereby saving time and associated labour costs.

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